PROTEIN PURIFICATION

This is a non-provisional application claiming priority under 35 USC §119 to provisional application No. 60/354,579 filed Feb. 5, 2002, the entire disclosure of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates generally to protein purification. In particular, the invention relates to a method for purifying C_H2/C_H3 region-containing proteins, such as antibodies and immunoadhesins, by Protein A affinity chromatography.

2. Description of Related Art

The large-scale, economic purification of proteins is increasingly an important problem for the biotechnology industry. Generally, proteins are produced by cell culture, using either mammalian or bacterial cell lines engineered to produce the protein of interest by insertion of a recombinant plasmid containing the gene for that protein. Since the cell lines used are living organisms, they must be fed with a complex growth medium, containing sugars, amino acids, and growth factors, usually supplied from preparations of animal serum. Separation of the desired protein from the mixture of compounds fed to the cells and from the by-products of the cells themselves to a purity sufficient for use as a human therapeutic poses a formidable challenge.

Procedures for purification of proteins from cell debris initially depend on the site of expression of the protein. Some proteins can be caused to be secreted directly from the cell into the surrounding growth media; others are made intracellularly. For the latter proteins, the first step of a purification process involves lysis of the cell, which can be done by a variety of methods, including mechanical shear, osmotic shock, or enzymatic treatments. Such disruption releases the entire contents of the cell into the homogenate, and in addition produces subcellular fragments that are difficult to remove due to their small size. These are generally removed by differential centrifugation or by filtration. The same problem arises, although on a smaller scale, with directly secreted proteins due to the natural death of cells and release of intracellular host cell proteins in the course of the protein production run.

Once a clarified solution containing the protein of interest has been obtained, its separation from the other proteins produced by the cell is usually attempted using a combination of different chromatography techniques. These techniques separate mixtures of proteins on the basis of their charge, degree of hydrophobicity, or size. Several different chromatography resins are available for each of these techniques, allowing accurate tailoring of the purification scheme to the particular protein involved. The essence of each of these separation methods is that proteins can be caused either to move at different rates down a long column, achieving a physical separation that increases as they pass further down the column, or to adhere selectively to the separation medium, being then differentially eluted by different solvents. In some cases, the desired protein is separated from impurities when the impurities specifically adhere to the column, and the protein of interest does not, that is, the protein of interest is present in the "flow-through."

Affinity chromatography, which exploits a specific interaction between the protein to be purified and an immobilized capture agent, may also be an option for some proteins. Protein A is a useful adsorbent for affinity chromatography

of proteins, such as antibodies, which contain an Fc region. Protein A is a 41 kD cell wall protein from *Staphylococcus aureas* which binds with a high affinity (about 10^{-8}M to human IgG) to the Fc region of antibodies.

Proteins may be purified using controlled pore glass (Sulkowski, E. *Protein Purification: Micro to Macro*, pgs 177–195 (1987); Chadha et al. *Preparative Biochemistry* 11(4):467–482 (1981)) or underivatized silica (Reifsnyder et al. *J. Chromatography* 753:73–80 (1996)).

U.S. Pat. Nos. 6,127,526 and 6,333,398 (Blank, G.) describe an intermediate wash step during Protein A chromatography using hydrophobic electrolytes,e.g., tetramethylammonium chloride (TMAC) and tetraethylammonium chloride (TEAC), to remove the contaminants, but not the immobilized Protein A or the protein of interest, bound to the Protein A column.

SUMMARY OF THE INVENTION

The present invention provides various intermediate wash buffers, other than TMAC or TEAC, for use in Protein A chromatography.

In one embodiment, the invention provides a method for purifying a protein which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising: (a) adsorbing the protein to Protein A immobilized on a solid phase; (b) removing contaminants by washing the solid phase with a composition comprising detergent and salt; and (c) recovering the protein from the solid phase.

In another embodiment, the invention provides a method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising: (a) adsorbing the protein to Protein A immobilized on a solid phase; (b) removing contaminants by washing the solid phase with a composition comprising a buffer at a concentration of greater than about 0.8M; and (c) recovering the protein from the solid phase.

The invention also pertains, in another embodiment, to a method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising: (a) adsorbing the protein to Protein A immobilized on a solid phase; (b) removing contaminants by washing the solid phase with a composition comprising salt and solvent; and (c) recovering the protein from the solid phase.

Moreover, the invention provides a method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising: (a) adsorbing the protein to Protein A immobilized on a solid phase; (b) removing contaminants by washing the solid phase with a composition comprising salt and polymer; and (c) recovering the protein from the solid phase.

In preferred embodiments, the protein is an antibody (e.g. one which binds HER2, vascular endothelial growth factor (VEGF), IgE, CD20, CD40, CD11a, tissue factor (TF), prostate stem cell antigen (PSCA), interleukin-8 (IL-8), epidermal growth factor receptor (EGFR), HER3, HER4$\alpha 4\beta 7$ or $\alpha 5\beta 3$) or an immunoadhesin (e.g. a TNF receptor immunoadhesin).

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A–B show the light chain amino acid sequence (SEQ ID NO:1) and heavy chain amino acid sequence (SEQ ID NO:2) of Trastuzumab (HERCEPTIN®).